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COMPREHENSIVE
CLINICAL TRIALS UNIT

Sub-study: The effect of prior exposure to the Measles, Mumps and Rubella vaccine on innate and adaptive immune responses to a SARS-CoV-2 mRNA vaccine

STATISTICAL ANALYSIS PLAN

VERSION 1.2, 21 September 2021

Protocol version	**2.0 dated 28 Feb 2021**
Clinicaltrials.gov	**NCT04646239**

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ABBREVIATIONS

AE	Adverse Event
BCG	Bacille Calmette-Guerin
CCTU	Comprehensive Clinical Trials Unit
COVID-19	SARS-CoV-2-induced disease
DMC	Data Monitoring Committee
EC50	Half-maximum effective concentration
IC50	Half-maximum inhibitory concentration
IFN	Interferon
IL	Interleukin
ITT	Intention-To-Treat
MMR	Measles, Mumps and Rubella vaccine
NK	Natura Killer
OPV	Oral Polio Vaccine
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RPMI	Roswell Park Memorial Institute
SAE	Serious Adverse Event
SARS-CoV-2	Virus causing COVID-19
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF	Tumor Necrosis Factor
TSC	Trial Steering Committee
UCL	University College London
WHO	World Health Organisation

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1 INTRODUCTION

1.1 General principles of the Statistical Analysis Plan (SAP)

This document details the proposed analysis strategy for the sub-study of CROWN CORONATION, leading to scientific publication(s). The results reported in these papers will follow the strategy set out here, which adheres to the guidelines for the content of a statistical analysis plan[1]. Moreover, the reporting of the main trial will adhere to the CONSORT guidelines[2].

Suggestions for subsequent analyses by oversight committees, journal editors or referees, will be considered carefully in line with the principles of this analysis plan. Subsequent analyses of a more exploratory nature will not be bound by this strategy, and will be detailed in separate analysis plans.

Any deviations from the SAP will be described and justified in the final reports. This document is intended to be stand-alone from the protocol and adhere to the main points in the analysis summary specified in the protocol. However, the SAP can undergo revision outside of the protocol.

1.2 Background and rationale of the sub-study

Adaptive memory immune responses (particularly B cells and antibodies) are considered the cornerstone of vaccination in humans. However, recent studies demonstrate that a memory-like response in innate immunity can also enhance resistance to infection. These observations have led to the paradigm-shifting concept of trained immunity, where functional reprogramming of innate immune cells such as monocytes, macrophages, and Natural Killer (NK) cells can improve immune responses against infections. Epidemiological studies have shown that certain vaccines employing live attenuated microorganisms such as Bacille Calmette-Guerin (BCG), measles-containing vaccines (such as MMR), and oral polio vaccine (OPV) exert heterologous protective effects against infections other than those for which these vaccines are administered, and this cannot be explained by induction of any specific T- or B cell responses. Vaccination with measles seems to induce a transient suppression of lymphoproliferative responses, but an increase in innate immune responses, as measured by nonspecific cytokine production.

Recent exposure to a vaccine may affect the immune response to subsequent vaccination with an unrelated vaccine. Such interaction between different vaccines has not been studied extensively. Evidence suggests that the chronological order of different vaccines in childhood immunization schedules can have a notable impact on overall childhood mortality. Interaction between vaccines may occur through modification of the adaptive response and / or modification of the innate immune response. Such interaction can be either positive or negative.

The rapid development and roll-out of specific SARS-CoV-2 mRNA vaccination in the USA,

coincided with enrolment of participants into the CROWN CORONATION trial (NCT04333732) – a study investigating the efficacy of the MMR vaccine to prevent symptomatic, PCR positive COVID-19. Subsequent to randomization to either MMR or placebo injection, a number of participants in the CROWN CORONATION trial were vaccinated with a SARS-CoV-2 mRNA vaccine.

1.3 Objectives of the sub-study

The goal of this sub-study is to characterize and compare, in those exposed to a) recent MMR vaccination followed by SARS-CoV-2 vaccination and b) recent placebo injection followed by SARS-CoV-2 vaccination,

I) the in-vitro, cytokine and chemokine responses (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-22, interferon (IFN)-α, and IFN-γ) to heterologous stimuli.

II) the SARS-CoV-2 neutralization assay (half-maximum effective concentration [EC50]; half-maximum inhibitory concentration [IC50]).

2 STUDY METHODS

2.1 Trial design

This is a sub-study within the CROWN CORONATION trial.

At Washington University in St Louis, consenting participants are recruited into the sub-study. Additional blood samples will be required from participants in the sub-study, to enable measurement of the outcomes of the sub-study. Apart from additional blood samples that will be collected from those in the sub-study, these participants will be treated the same as those taking part in the CROWN CORONATION trial.

2.2 Framework

CROWN CORONATION is a superiority trial. The aim is to demonstrate that the trial intervention(s) is(are) effective at decreasing the incidence of symptomatic COVID-19 disease.

In the sub-study, we hypothesize that, compared to SARS-CoV-2 mRNA vaccination alone, MMR vaccination prior to SARS-CoV-2 mRNA vaccination will induce lasting trained immunity in recipients, manifested by improved innate immune responses upon in vitro exposure of supernatants to heterologous pathogen products. We also hypothesize that MMR vaccination prior to SARS-CoV-2 mRNA vaccination does not alter the adaptive immune response to SARS-CoV-2 mRNA vaccination.

2.3 Randomisation

Sequence generation, allocation and implementation takes place within the CROWN CORONATION trial.

2.4 Sample size

All CROWN CORONATION participants who meet the inclusion criteria will be contacted for recruitment. We will enrol as many participants as possible into the sub-study. The eligible population size is 160.

For the purpose of sample size calculation, we used the t-test to compare the primary outcome between treatment arms, i.e. the difference in cytokine and IFN-γ response between MMR and placebo groups. Table 1 reports power given the expected difference in IFN-γ responses, with a two-sided type-1 error rate of 5%.
Table 1: Predicted power.

Stimulus	Expected difference between means (pg/ml)	Expected standard deviation	Power with 60 participants	Power with 100 participants	Power with 140 participants
TLR-3 ligand	400	900	39%	59%	74%
TLR-7/8 ligand	450	900	48%	70%	84%

2.5 **Study outcomes**

i) Primary outcomes:

a. PBMC cytokine production (TNF-α, IL-1β, IL-6, and IFN-α) 1 day after stimulation with heterologous products (inactivated SARS-CoV-2, live attenuated MMR viruses, toll-like receptor (TLR)-3 ligand, TLR-7/8 ligand, TLR-4 ligand, and RPMI medium).

b. IL-10, IL-17, IL-22, and IFN-γ production 5 days after stimulation of PBMCs with heterologous products.

ii) Secondary outcomes:

c. SARS-CoV-2 neutralization assay (EC50; IC50).

d. Measles IgG antibody titres

Timing: Blood collected at a single sub-study visit around 6 to 9 weeks following the participant's second SARS-CoV-2 mRNA vaccine injection.

3 STATISTICAL PRINCIPLES

This sub-study is hypothesis generating. No adjustment will be made for multiple testing. All results comparing the treatment arms will be provided with 95% confidence interval (except if specified otherwise). Analysis will be for complete cases; no imputation will be performed for missing data. Missing data will be summarised and reported with results.

3.1 Analysis population

All randomised participant data will be included in the Intention-To-Treat (ITT) analysis according to the arm they were randomised to, irrespective of the actual study drug that they took. This ITT analysis will be the main strategy for the sub-study (primary and secondary outcomes).

A modified ITT analysis will be performed at the end of the CROWN CORNATION trial, based on serology results. Participants with evidence of COVID-19 infection at the start of the study will be removed from the analysis set. It is anticipated that this sub-study will be completed and reported on prior to analysis of the main trial so that the modified ITT analysis of the sub-study data will be reported separately.

3.2 Baseline patient characteristics

Baseline characteristics will be summarised for all participants in the sub-study. Summary measures for the baseline characteristics will be presented as mean and standard deviation for continuous (approximate) normally distributed variables, medians and interquartile ranges for non-normally distributed continuous variables, and frequencies and percentages for categorical variables. We will plot histograms of continuous variables to assess normality.

3.3 Analysis methods

The results of the analyses will be reported following the principle of the ICH E3 guidelines on the Structure and Content of Clinical Study Reports[5]. There will be no imputations for missing data.

Endpoints will be summarised graphically with box-whisker-dot plots.

3.3.1 Adjustment factors

All models for the primary outcomes will be adjusted for sex and the calendar date of sample collection (by quarter of the year). Diagnostics for regression models will be assessed to ensure that underlying assumptions are met.

Sensitivity analyses will be performed by individually adding adjustment for age, interval duration between last SARS-CoV-2 vaccine injection and blood draw, interval duration between MMR or placebo injection and blood draw, and interval duration between MMR or

placebo injection and SARS-CoV-2 vaccination (figure 1). Outcomes will be plotted over time to assess the effect of the stated interval durations (figure 1) on the measured outcomes.

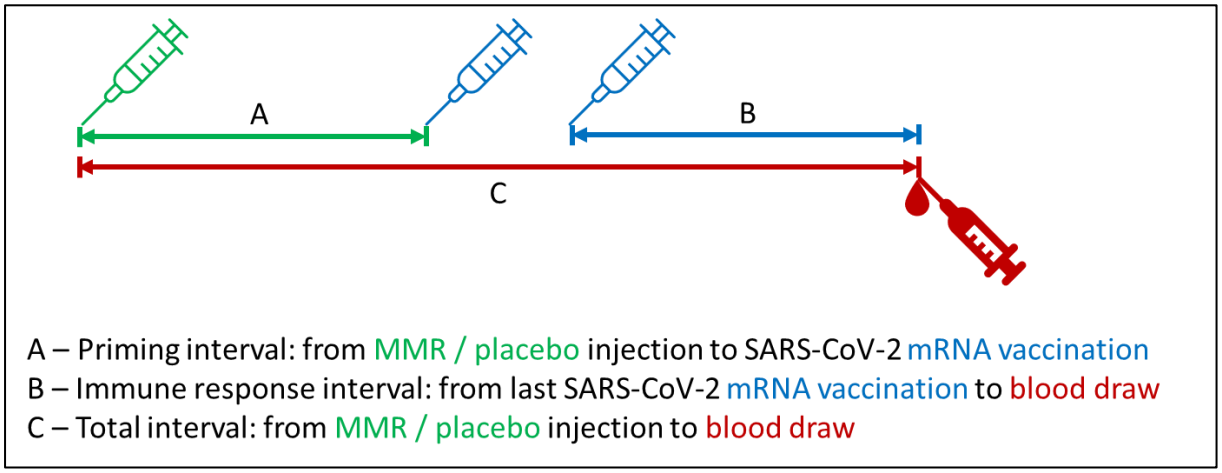

Figure 1: Interval durations in sub-study

3.3.2 Primary endpoints analysis

TNF-α, IL-1β, IL-6, IL-10, IL-17, IL-22, IFN-α and IFN-γ will be analysed by linear regression, with a coefficient for treatment arm, and adjusted for sex and the calendar date (which quarter of the year) of sample collection. The endpoints will be reported as the adjusted mean (95% CI) effect of MMR pre-exposure on the endpoint. For each specified endpoint, the individual participant assay response to RPMI will be evaluated and if the response is greater that three times the lower detectable limit of the assay, the data from that participant will be excluded from analysis for the specific endpoint. For example, if the IL-6 value is greater than three times the lower detectable limit in response to RPMI, the participant's data will be excluded from the analyses of IL-6 responses to the heterologous stimuli. The participant's data will still be used for the other endpoints in which the assay response to RPMI was less than three times the lower detectable limit.

3.3.3 Secondary endpoints analysis

SARS-CoV-2 neutralization assay (EC50; IC50) and Measles IgG antibody titres will be analysed by linear regression, with a coefficient for treatment arm, and adjusted for sex and the calendar date (which quarter of the year) of sample collection.

4 APPROVALS

Lead Trial Statistician	Name: Hakim-Moulay Dehbi	
	Signature: 	Date: 04 Oct 2021
Investigator	Name: Leon du Toit	
	Signature: 	Date: 30 Sep 2021

5 REVISION HISTORY

Version	Date	Edited by	Comments/Justification	Timing in relation to first unblinded analysis
0.1	27/07/2021	HMD	First draft	Prior
0.2	04/08/2021	LDT	Revision	Prior
1.0	10/08/2021	LDT	Version 1	Prior
1.1	15/09/2021	LDT	Revision	Prior
1.2	21/09/2021	HMD	Version 1.1	Prior